EPILEPTIFORM ACTIVITY INDUCTION WITH ELECTROLYTE IMBALANCE IN BRAIN SLICES: MECHANISMS INVOLVED IN CONTROL

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***Abstract* - Epilepsy is a disorder of recurrent seizure activity caused by rhythmic firing of neurons. Epileptiform activity can be generated by incubating brain slices in magnesium-free artificial cerebrospinal fluid (ACSF). In the present study, epileptiform discharges induced by the omission of magnesium ions from ACSF has been studied in hippocampal slices obtained from young rats using patch clamp tight-seal whole cell recording technique. Effects of AP5 to block NMDA receptor activation and acidic pH shift of ACSF on the epileptiform current was studied. It was found that magnesium-free ACSF induced epileptiform activity frequency was attenuated with AP5 application more than 50 %. The pH shift of the magnesium-free ACSF from 7.3 to 7.1 depressed the epileptiform activity. Both effects were shown to be reversible. According to the results of this study, epileptiform activity and mild extracellular acidic shifts do not interact to aggravate excitotoxicity conditions in CA1 pyramidal neurons.**

***Key words* - Hippocampal slices, Patch clamp recording, Epileptiform activity, CA1 pyramidal neuron, NMDA receptor**

I. INTRODUCTION

Epilepsy is a disorder of recurrent seizure activity that is caused by synchronous and rhythmic firing of neurons. It can be caused by the deficiency of certain chemicals. Seizure occurring in immature brain is generally symptomatic; that is due to the electrolyte imbalance. These imbalances may include calcium, magnesium, glucose, amino acid and pyridoxine deficiencies. The hippocampal formation is located in temporal lobe and shown to be involved in epilepsy. The relation between the hippocampal sclerosis, temporal lobe epilepsy and the development of childhood seizure has been shown in affected individuals [1]. Profound CA1 pyramidal cell loss was observed in patients suffering from uncontrolled temporal lobe epilepsy. In the same patients, by intracranial recordings, the loci for the generation of the ictal spikes have been reported to be the CA1 pyramidal cell region [2,3].

The amino acid glutamate is known to be an important neurotransmitter in the CNS to evoke the neuronal excitation and also a potent neurotoxin [4]. The involvement of the pathological activation of glutamate receptors in epileptic brain damage in rat hippocampus has been shown [5]. Glutamate release from the presynaptic neuron excites different receptor subgroups that have distinct pharmacological and anatomical distributions [6,7]. These receptor subgroups are; N-methyl-D-aspartic acid (NMDA), α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate and metabotropic receptors. NMDA receptor-channel complex is a coincidence detector; it is activated by binding of glutamate to the agonist site of the receptor and with omission of magnesium ions from the channel in a voltage dependent way [8,9,10]. NMDA channel is permeable to sodium, potassium, and calcium ions. Selective calcium ion permeability of NMDA channel is believed to be important in physiological and pathological events occurring in neuron [11,12]. In low magnesium containing media NMDA channel loses its voltage sensitivity and becomes permeable at any membrane voltage [8]. In human epileptogenic neocortex slices perfusion with magnesium-free artificial cerebrospinal fluid (ACSF) caused the generation of spontaneous epileptiform activity that was effectively blocked by NMDA receptor antagonist (±)-2-Amino-5-phosphonopentanoic acid (AP5) [13]. Magnesium ion is a physiological necessity and involved in enzymatic reactions, which modulates the functioning of potassium, calcium channels and NMDA receptor-ionophore complex [8,9,14,15]. In CA1 pyramidal neurons wash with magnesium-free ACSF did not abolish the inhibitory synaptic potentials, the enhancement of the activity was rather due to increased excitation [16].

Hippocampal CA1 region is rich in NMDA receptors and very sensitive to extracellular pH changes [17,18]. Extracellular pH is highly dynamic in mammalian brain and influences functioning of biochemical processes, proteins and receptors. In hippocampal slices obtained from young animals, the susceptibility of the CA1 region to pH changes was found to be higher than CA3 and dentate gyrus [19].

In the present study, enhanced excitation model for the induction of the epileptiform activity has been used in the absence of synchronized external stimulation. Under this condition, epileptiform currents were recorded from CA1 region of hippocampal slices by using patch clamp tight-seal whole cell recording technique. The effect of selective NMDA antagonist AP5 and the influence of mild acidic shift on the epileptiform activity in the vulnerable CA1 region have been studied.

II. MATERIALS AND METHODS

Hippocampal slices of 300 μm thickness were obtained from young Sprague-Dawley rats (14-21 day old) by using a vibroslicer (Campden Instrument LTD). Hippocampal slices

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were incubated in ACSF, at room temperature for functional recovery. Hippocampal slice preparation offers the advantage of studying organized and intact synaptic structures under *in vitro* conditions [20].

The ACSF contained (in mM:): 125 NaCl; 3.75 KCl; 26 $NaHCO_3$; 1.2 NaH_2PO_4; 1.3 $MgCl_2$; 2 $CaCl_2$; 10 Glucose. Magnesium free ACSF was not containing magnesium ions. Both solutions were continuously bubbled with carbogen. The control current recordings were made under ACSF perfusion of slices, then perfusion solution was changed to magnesium free ACSF. NMDA receptor antagonist AP5 (60 μM) was applied to the Mg^{2+}-free ACSF. Patch clamp tight-seal whole cell recordings were made from CA1 stratum pyramidale region of the hippocampal slices. The patch electrodes were positioned under the visual control to the stratum pyramidale region. The tip resistance of the patch electrodes were ranging between 3-5 MΩ. Positive pressure was applied to the recording electrode before insertion into the slice. With an approach to the CA1 pyramidal cell the test current decreased. Following the release of the positive pressure the subsequent suction facilitated the giga-seal formation. Upon the breakthrough to the whole cell mode, the cells having a resting membrane potential of -60 mV or lower with no spontaneous discharge were accepted. The recorded activity was spontaneous activity; thus there was no synchronized external stimulation to any of the hippocampal tracts.

Acidic condition was mimicked by acidic pH shift of 0.2 unit of the perfusion solution that hippocampal slices were bathed while patch clamp recording from the CA1 pyramidal cell was continued. The pH of the perfusion system was 7.3-7.4. Reported pH values reflect the values for the gassed reservoir of Mg^{2+}-free ACSF. Recordings were made in continuous voltage clamp mode.

Acquired data were processed using the MATLAB software (version 5.0). Baseline of traces in Fig.1 and 3 were fixed at zero pA to provide the consistency in y-axis. Histograms were computed from 15 waveforms yielding a total of 1 minute analysis period.

III. Results

The current recordings were made from CA1 pyramidal cells of young rats with stable membrane potentials of -60 mV or lower in ACSF. Upon the change of ACSF to Mg^{2+}-free ACSF, the epileptiform activity was initiated. The epileptiform current activity was reverted to the control activity upon the change of the Mg^{2+}-free ACSF to ACSF.

Effect of AP5 (60 μM) on the epileptiform activity is shown in Fig. 1. The control activity under the Mg^{2+}-free ACSF perfusion (Fig. 1A) was reversibly depressed with AP5 application (Fig. 1B). The observed spike occurancy depression upon AP5 application in different CA1 pyramidal neurons were more than 50 % (Ave= 69.5; SEM= ±3.7)(n= 8). In some of the neurons AP5 application caused the complete cessation of epileptiform activity (n=3). Histograms were constructed from the AP5 application data for one minute of continuous recording in each condition and show the depression of epileptiform current occurrence (Fig. 2).

Mild acidity was mimicked by extracellular pH shift of Mg^{2+}-free ACSF. Epileptiform discharges were recorded at pH 7.3 (Fig. 3A). With the change of the perfusion solution to Mg^{2+}-free ACSF having pH 7.1, the frequency of activity was depressed (Fig. 3B). The histograms of pH effect were constructed from one minute of continuous recording in each condition and show the decrease in the number of occurrence and amplitude of currents (Fig.4). The effect of increased proton concentration on the epileptiform current was reversible.

IV. Discussion

In the present study, epileptiform discharges induced by the omission of magnesium ion from ACSF have been studied in hippocampal slices. The suppressant effect of NMDA receptor antagonist AP5 on the epileptiform current frequency has been shown. Furthermore, the depressant effect of acidic pH shift on the epilepsy activity has been demonstrated.

Magnesium ion deficiency induces depolarization and hyperexcitability in hippocampal neurons that may carry the neuron to the threshold of excitotoxicity [16]. The depressant action of anticonvulsants on magnesium-free ACSF induced epileptiform activity has been shown before [21]. Magnesium ion modifies the functioning of potassium, calcium channels and NMDA receptors [8,9,14,15]. Peripheral administration of the magnesium sulfate increased the magnesium concentration in the cerebrospinal fluid and the uptake has been reported to be the highest in the hippocampus [22]. In our study, we showed the effect of the magnesium ion deficiency in hippocampal slices of young rats under the most basal activity; i.e., the spontaneous activity condition.

As the experimental results of this study indicated, epileptiform activity was downregulated with extracellular acidic shift of 0.2 pH unit; a change that has physiological relevance. pH changes are well documented in central nervous system during synaptic transmission, glutamate receptor activation, ischemia and seizures. It has been reported that the cerebral blood flow reduction is followed by a subsequent acidic change of 0.3 pH unit in extracellular pH [23]. Under pathological conditions like ischaemia and pH decrease, conditions for the excessive activation of the neurons are available. Metabolic energy production is impaired and glutamate is abundantly present in the extracellular space. The excessive stimulation of the glutamate receptors may cause the disturbance of calcium and sodium ions homeostasis. The importance of the calcium ion in the initiation of the neuronal excitotoxicity has been shown [24].

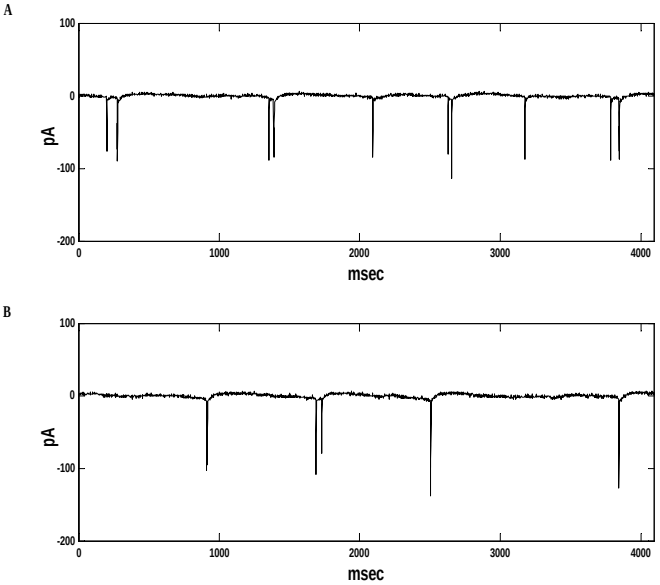

Fig. 1. Effect of the competitive NMDA antagonist AP5 on the epileptiform activity recorded from CA1 pyramidal neuron. A) Epileptiform current frequency was 2.5 Hz with magnesium free ACSF perfusion. B) Upon AP5 (60 μM) application to the magnesium free ACSF the epileptiform activity decreased to 1.2 Hz. The epileptiform current occurrence was depressed by 50 % in this neuron.

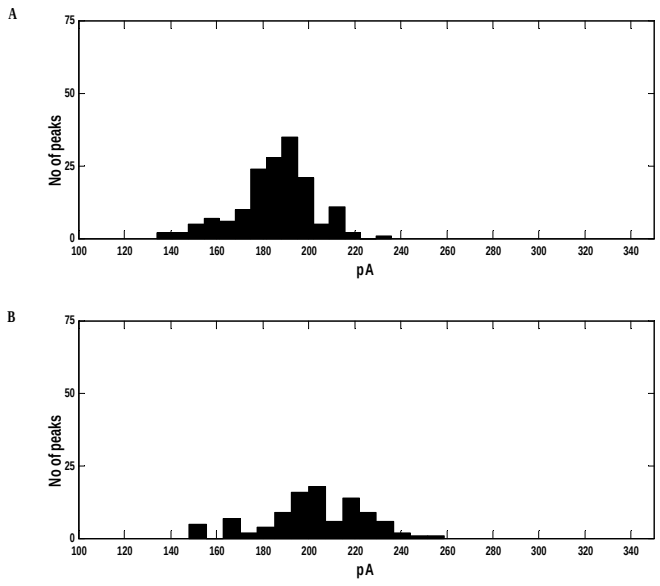

Fig. 2. Histograms show the effect of the AP5 application on the epileptiform activity. Data obtained from one minute recording were used to draw histograms. A) Epileptiform current distribution during perfusion with magnesium free ACSF. B) Application of AP5 decreased the occurence of the spontaneous epileptiform currents.

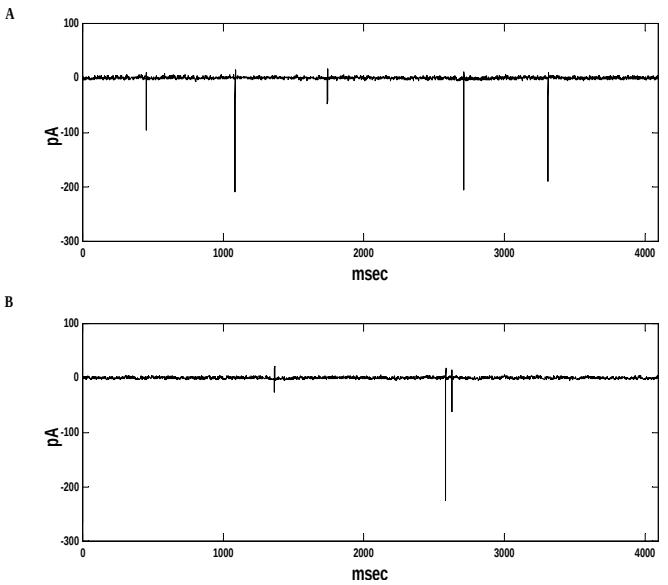

Fig. 3. Effect of extracellular acidic shift (0.2 pH unit) on epileptiform current recorded from CA1 pyramidal neuron. A) The control epileptiform current recorded at pH 7.3; the discharge frequency was 1.2 Hz. B) Epileptiform current frequency was depressed to 0.75 Hz by changing perfusion solution to magnesium free ACSF having pH 7.1.

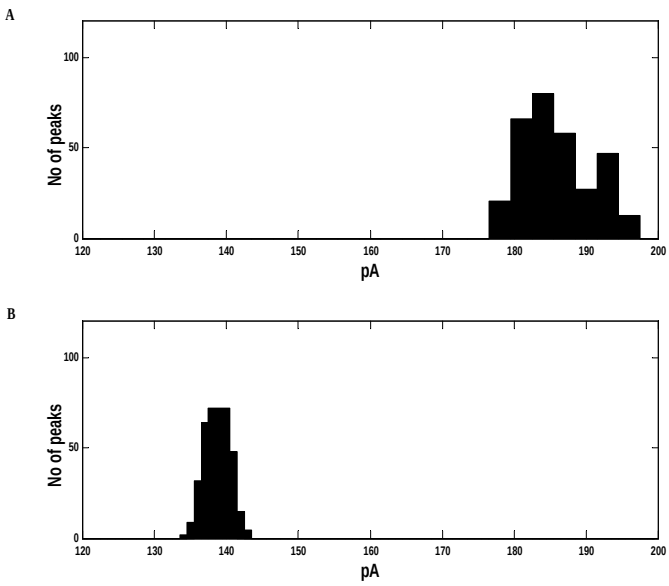

Fig. 4. Histograms show the effect of 0.2 unit pH change on epileptiform activity. Data obtained from 1 minute of continuous recording were used in each condition. A) Epileptiform current distribution of CA1 pyramidal cell during perfusion with magnesium free ACSF (pH 7.3). B) Changing the perfusion solution (pH 7.1), decreased the number of occurrence and amplitude of spontaneous epileptiform currents.

Exposure to high concentrations of glutamate caused mitochondrial depolarization, superoxide radical generation and neurotoxicity with the activation of NMDA receptors in cultured forebrain neurons [25,26]. Superoxide radical generation was increased in hippocampal slices incubated in magnesium-free ACSF (our unpublished results), indicating that conditions leading to excitotoxicity are initiated with magnesium free model of epilepsy.

In this study, magnesium-free ACSF induced epileptiform activity of CA1 hippocampal pyramidal cells were depressed with mild acidic shift. Likewise, as an intrinsic protection mechanism the selective depressant effect of increased proton concentration on NMDA current has been reported in CA1 pyramidal cells of hippocampal slices [27], in dissociated cell culture of hippocampal neurons [28] and in cerebellar neurons [29]. It is possible that the presence of excessive neuronal activation that might be leading to the neuronal damage is not exacerbated but controlled with mild extracellular acidic shifts. Extracellular acidity seems to initiate a hindrance mechanism to prevent the excessive neuronal damage caused by epileptiform activity conditions in hippocampal CA1 pyramidal neurons of young rats.

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